

Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) **EP 0 752 245 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention  
of the grant of the patent:  
22.05.2002 Bulletin 2002/21

(51) Int Cl.<sup>7</sup>: **A61K 9/51**

(21) Application number: **95110445.4**

(22) Date of filing: **05.07.1995**

(54) **Biocompatible and biodegradable nanoparticles designed for proteinaceous drugs absorption and delivery**

Biokompatible und biodegradierbare Nanokapseln zur Absorption und Verabreichung von  
proteinischen Arzneimitteln

Nanoparticules biocompatibles et biodégradables conçues pour l'absorption et l'administration de  
médicaments protéiniques

(84) Designated Contracting States:  
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL  
PT SE**  
Designated Extension States:  
**LT LV SI**

(56) References cited:  
**EP-A- 0 275 796 EP-A- 0 520 888**  
**EP-A- 0 529 711 EP-A- 0 661 055**  
**WO-A-88/08011 WO-A-91/15193**  
**US-A- 5 330 768**

(43) Date of publication of application:  
**08.01.1997 Bulletin 1997/02**

(73) Proprietor: **EUROPEAN COMMUNITY**  
**2910 Luxembourg (LU)**

(72) Inventors:  
• **Grandfils, Christian**  
**4020 Jupille (BE)**  
• **Jerome, Robert**  
**4130 Tilff (BE)**  
• **Nihant, Nicole,**  
**4651 Battice (BE)**  
• **Teyssie, Philippe,**  
**Neuville en Condroz (BE)**

(74) Representative: **Godemeyer, Thomas, Dr.**  
**Sternagel, Fleischer, Godemeyer & Partner**  
**Patentanwälte**  
**An den Gärten 7**  
**51491 Overath (DE)**

- **J. OF PHARMACEUTICAL SCIENCES**, vol. 79,  
no. 9, 1990 pages 763-767, **AMMOURY N. ET AL**  
'In Vitro Release Kinetic Pattern of Indomethacin  
from Poly(D,L-Lactide) Nanocapsules'
- **EUROPEAN J. OF PHARMACEUTICS AND**  
**BIOPHARMACEUTICS**, vol. 41, no. 1, January  
1995 **STUTTGART**, pages 2-13, **XP 000482885**  
**COUVREUR P. ET AL** 'Controlled Drug Delivery  
with Nanoparticles: Current Possibilities and  
Future Trends'
- **CONGR. INT. TECHNOL. PHARM.**, vol. 2, 1992  
**CHATENAY MALABRY**, pages 308-326, **REICH**  
**G.** 'Preparation and Characterization of  
Indomethacin-Loaded Nanoparticles Using  
Poly(DL-Lactide)/Synperonic Blends'

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

**EP 0 752 245 B1**

## Description

[0001] The invention relates to nanoparticles for proteinaceous drugs or peptides, to a process for the preparation of nanoparticles and to the use of nanoparticles for the manufacture of a medicament for controlled release drug delivery systems.

[0002] Nanoparticles in form of pseudolatex are derived from an oil-in-water submicron emulsion that is converted to colloidal polymer dispersion after removal of the organic solvent, used to dissolve the preformed polymer. Thus, they differ from most common latex prepared from water insoluble monomers by emulsion or dispersion polymerization. The major advantage of pseudolatexes relies upon the feasibility to convert most polymers into an organic colloidal dispersion allowing direct incorporation of plasticizers. Pseudolatexes of ethylcellulose in celluloseacetatephthalat have been used for pharmaceutical coating applications (Banker et al., *Pharmaceutical Technology* 1981, pp 55 - 61).

[0003] Nanocapsules of polylactide of size centered around 220 nm were reported Amnoury in *J. Pharm. Sci.*, 1990, pp 763 - 767. The nanocapsules were prepared by an interfacial deposition technique. This method was applied for encapsulation of indomethacin.

[0004] Nanoencapsulation of low molecular weight antibiotic in polylactide-glycolide material was reported by Julienne et al., *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.*, 1989, pp 77 - 78.

[0005] Bazile et al. reported in *Biomaterials* 1992, pp 1093 - 1102 about the preparation of nanoparticles of polylactide (90 to 250 nm) coated with albumin. The protein was used as stabilizer of the oil-in-water emulsion without mentioning any encapsulation of an active drug and its sustained release.

[0006] Gref et al. in *Science* 1994, 263, pp 1600 - 1603 have described the formulation of polylactide (or polycaprolactone)-polyethyleneglycol block copolymer in 140 nm pseudolatexes. The amphiphilic copolymers has been used to encapsulate low molecular weight compounds that are released in some hours in vivo.

[0007] US 5,330,768 describes a drug delivery system using polymer/pluronic blends. As polymers polylactide and non-ionic pluronic surfactants like block-copolymers of polyethylene oxide and polypropylen oxide can be used. The blends are mentioned in order to control the drug release kinetics particularly of proteins.

[0008] Nanoparticles are used as drug delivery systems. In colloidal drug delivery systems, edited by Jörg Kreuter, Marcel Dekker, Inc., New York, Basel, Hong Kong, pp 219 - 341, the significance of nanoparticles is described as well as conventional methods for producing such nanoparticles. Biodegradable pseudo-latex particles (nanoparticles) have been prepared from biocompatible polyesters by conventional emulsification/solvent/solvent diffusion methods. The coating polymer is initially dissolved in a water immiscible chlorinated organic solvent (or in mixtures of chlorinated and more po-

lar solvents). Size of the particles usually exceeds 100 nm and no information is available about the effective encapsulation of especially protein drugs.

Although, nanoparticles have been proven to be useful tools for drug delivery systems. Some problems relating to the control of particle size as well as particle loading with protein drugs are not solved. Also a high loading, a safeguarding drug activity and allowing for controlled drug release is a feature which is hard to match.

[0009] The object of the present invention is to improve nanoparticles for the purpose of serving as drug delivery systems in particular for proteins or polypeptides.

[0010] This object is solved by nanoparticles with a particle size between 0.02 to 1  $\mu$ m as described in the claims.

[0011] The nanoparticles according to the invention can be prepared by the following steps :

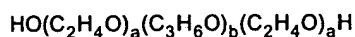
- mixing the biocompatible polymer and the biocompatible interacting agent in an organic solvent,
- mixing the solution and evaporating the solvent to form an essentially homogenous mixture or melting one of the components (the biocompatible polymer or the biocompatible interacting agent) and adding the other component (the biocompatible interacting agent or the biocompatible polymer, respectively) so that an essentially homogenous mixture is built up, or melting both components (biocompatible polymer and biocompatible interacting agent) together and forming an essentially homogenous mixture,
- dissolving the polymer blend in a water soluble organic solvent selected from the group consisting of dimethyl sulfoxide, acetone, tetrahydrofurane, 1,3-dioxolane, N,N-dimethylformamide or mixtures thereof,
- adding cholesterol or an anionic cholesterol derivative as biocompatible surfactant,
- adding a polypeptide and/or a protein as drug or drug combination, wherein these steps are carried out in such a manner that the mixture of polymer blend, biocompatible surfactant and drug or drug combination forms an essentially homogenous mixture,
- contacting the mixture with an aqueous phase to form a dispersion and
- separating the formed nanoparticles from the liquid phase.

[0012] Preferably, the biocompatible polymer is a polymer which can be readily melted or used in a solvent deposition process for preparing nanoparticles. If the polymer can be readily melted this is quite convenient since it must not be dissolved and can be directly mixed intimately with the biocompatible interacting agent. During the melting process the polymer should not be decomposed or start to decompose.

[0013] When the biocompatible polymer is to be dis-

solved, preferably organic solvents are used. In particular, the biocompatible polymer is an aliphatic polyester such as poly-ε-caprolactone, polylactide or polyglycolide, or a copolymer thereof. Preferably, poly(D,L)lactide or poly(D,L)lactide-co-glycolide are used in particular in a 50% : 50% molar ratio. The polymer molecular weight is preferably in the range from 10.000 to several hundreds of thousand in particular from 20.000 to 50.000.

[0014] The biocompatible interacting agent is a polymer containing alcohol, ether and/or ester constitutive units. Preferably, the biocompatible interacting agent is either a polyether or polyvinyl alcohol. The preferred polyether is a polyethylene glycol or a polyoxyethylene-polyoxypropylene-polyoxyethylene triblock copolymer having in particular the general formula:



wherein the lit a and b are selected to give molecular weight of the polyethers in the range of some hundreds to 20.000, preferably about 8.500, preferably lit a = 75 and b = 30. Such compounds are named poloxamers. Typically, the biocompatible interacting agent is present in the polymer blend composition between 10 and 60% by weight, preferably about 50% by weight.

[0015] The biocompatible interacting agent can be also an amphiphilic graft copolymer containing an hydrophilic backbone grafted with hydrophobic aliphatic polyester subchains. The synthesis of these copolymers has been previously described by Barakat et al. (J. Polym. Sci. A. Polym. Chem. 1994, 32, 2099-2110). The hydrophilic/lipophilic balance of the copolymer can be controlled by the comonomer molar ratio, the molecular weight of polylactide chains and the nature of the hydrophilic comonomer, e.g. polyhydroxyethylmethacrylate (HEMA), acrylamide, and N-vinyl-pyrrolidone. These copolymers can be directly dissolved together with the biocompatible polymer so that the preliminary step of blending may be avoided.

[0016] The polymeric blend of the invention is used to prepare the nanoparticles of the present invention. The polymer blend is an intermediate product which is manufactured according to the following process. The biocompatible polymer is for example melted together or prior to the addition of the interacting agent. The two components are thoroughly mixed and the mixture is then ready to be used for preparing the nanoparticles of the invention.

[0017] Alternatively, the components are dissolved in an organic solvent and poured together under mixing. After combining the solution the solvent is evaporated. For example, the polymers are simultaneously dissolved in an organic solvent preferably acetone or a chlorinated solvent at room temperature. The concentration of each polymer is in the range of 1 to 10% by weight, preferably about 3 to 5% by weight. The solvent can be evaporated under a nitrogen flux at ambient tem-

perature and subsequently at 40°C until dryness.

[0018] The dissolution of the polymer blend of the invention can be performed at room temperature during a time range of about 30 min. A concentration range of the polymer blend is preferably in the range of 0.225 to 5% by weight, more preferred about 0.3 to 0.7% by weight. According to the process of the invention the biocompatible surfactant is an anionic derivative of cholesterol particularly cholesterol 3-sulfate. The surfactant in the oily phase of the dispersion is in the range of 0.01 to 0.2% by weight, preferably about 0.05% by weight. The surfactants are preferably low range hydrophilic/lipophilic balanced compounds (HLB).

[0019] The drug which can be used according to the invention is a low molecular lipophilic compound or polypeptide. For example, a low molecular weight drug such as diazepam or antibiotics, anesthetics or antimitotics can be taken into account. The polypeptide could be a peptide or protein including enzymes or antigen molecules such as bovine serum albumin (BSA) or somatotropin or tetanus toxoid, influenza virus. Also drug combinations can be used in the process of invention, for example, diazepam/somatotropin.

[0020] The drug loading depends on the nature of the substance and is typically in the range of 0.2 to 20% by weight based on the amount of polymer, preferably about 10% by weight. Depending on the solubility and stability of the drugs to be loaded to the nanoparticles they can be initially solubilized either in water before addition in the organic solution or directly in the organic solution.

[0021] Typically, the two phase system in which the nanoparticles are formed is consisting of an oil/aqueous phase. The volume ratio is about 0.7 to 15%, probably 8 to 12%. The dispersion is typically performed using machines e. g. stirrer, rotor-stator equipment or high pressure homogenizer. Preferred is a rotor-stator equipment. The dispersion is carried out at room temperature for 15 sec. to 2 min. The preferred organic solvents are water soluble organic solvents such as DMSO, acetone, tetrahydrofuran, 1,3-dioxolane, N,N-dimethylformamide or mixtures thereof.

[0022] The process of the invention can be controlled by changing the polymer concentration in the oil phase, the oil-aqueous phase ratio, the surfactant and its concentration in the oil phase and solvent itself.

[0023] Also the composition of the polymer blend (weight ratio of the two polymers) has an effect on the average particle size. According to the invention the process conditions are adjusted that nanoparticles of a size of 0.02 to 1 μm, preferably 50 to 200 nm (average size) are formed. In the process of the invention the separation of nanoparticles in the liquid phase is achieved by filtration or centrifugation. The nanoparticles are separated from the original liquid phase in order to eliminate the organic solvent(s) and the non-encapsulated drug. Preferably, the filtration is carried out at 25°C with an ultrafiltration membrane, preferably by tangential-ultra-

filtration in order to avoid membrane blocking. A suitable membrane is, for example, a cellulosic type membrane of a spiral-type configuration within 0.09 M<sup>2</sup> area and a cut off of 100.000. After a first diafiltration cycle nanoparticles are diluted e. g. 2 l of water and purified twice so as to eliminate up to 99.9% of the original solvent. The organic solvent can also be removed from nanoparticles by extraction into the aqueous phase and or ultrafiltration or by evaporation if the organic solvent is sufficiently volatile. Centrifugation is an alternative to the latex filtration when the particles settle down easily without any aggregation.

[0024] It may be advantageous to add a polymer such as polyethyleneglycol and/or a polyoxylene-polyoxylene triblock copolymer for making easier redispersion of the nanoparticles after they have undergone a freeze-drying. The additive concentration in latex suspension is in a range of 2 to 10% by weight, preferably 3 to 5% by weight.

[0025] The invention allows production of biocompatible and biodegradable pseudolatex particles with a homogenous size below 140 nm. The nanoparticles of the invention advantageously show efficient immobilization of proteinaceous drugs by preserving their activity and allowing for their controlled release. The nanoparticles of the invention are stable colloidal carriers against environmental attacks such as pH modifications or freezing or freeze-drying processes. All excipients of the formulation are biocompatible for parenteral uses. The polymer blend of the present invention is used for the preparation of colloidal vectors. The polymer blend of the invention has specific properties in terms of solubility, viscosity and in terms of protein interaction. The nanoparticles prepared by the polymeric blend of the invention can advantageously be used for administering proteinaceous drugs. By using the polymeric blend of the invention the activity of the immobilized protein can be kept.

[0026] The nanoparticles of the invention can be applied in methods of treating patients with drugs or the drug combinations. Thereby, the drugs or drug combinations are adhered on/or incorporated in nanoparticles. They are administered to patients in need of the drugs associated with the nanoparticles orally, intraperitoneally, topically, intranasally, intravenously in amounts to give an active dose. Examples of treatments are clinical applications such as diabetes and tumor treatment, inflammation and intracellular infection treatment (ophthalmic delivery e.g. pilocarpine, anti tumor drugs and so on), targeting towards inflammation in the body, adjuvant for vaccine, vaccine controlled delivery (e.g. HIV virus or influenza).

[0027] Figure 1 shows the in vitro release of somatotropin (ST). The I-125 and radio-immuno-assay (RIA) curves correspond to the total and active form of the protein released respectively (cumulative curves).

[0028] Figure 2 shows the size distribution of PLGA-F68 nanoparticles measured by PCS.

[0029] Figure 3 shows a micrograph (TEM) of nanoparticles.

[0030] Figure 4 shows in vitro release of somatotropin (ST). The I-125 and RIA curves correspond to the total and active form of the protein released respectively (cumulative curves).

[0031] Figure 5 shows the in vitro release of somatotropin (ST). The I-125 and radio-immuno-assay (RIA) curves correspond to the total and active form of the protein released respectively (cumulative curves).

[0032] The invention is further explained by the following examples.

## EXAMPLES

### Example 1

#### Nanoparticles prepared from a poly(D,L)lactide-co-glycolide poloxamer blend and used to immobilize somatotropin

[0033] 225 mg of a poly (D,L)lactide-co-glycolide copolymer (50 mol %; Mn : 20,000; designated as PLGA) and 225 mg of a polyoxyethylene-polyoxypropylene-polyoxyethylene triblock copolymer (Poloxamer : Pluronic® F68) were dissolved in 5 ml of methylene chloride at room temperature for 15 min. The organic solvent was let to evaporate under nitrogen, at room temperature and then to 40°C. This polymer blend could be used immediately or stored at 4°C for several days under dry conditions.

[0034] Cholesterol 3-sulfate (25 mg) was added to the polymer blend followed by 50 ml of DMSO (p.a.). Dissolution took place for 25 min. at room temperature. Somatotropin (25 mg of a lyophilisate powder) was added to the organic solution and dissolved (5 min). This organic solution was dispersed for 2 min. in 700 ml of water with a rotor-stator equipment (Ultra-turrax; IKA Werke, Janke and Kunkel GmbH & Co. KG, Staufen, Germany; S25N-18g drive unit, 24,000 rpm). The suspension obtained was diluted by water until a total volume of 2 l, and then purified by ultrafiltration by using a tangential ultrafiltration equipment (Spiral membrane Amicon, Inc., Beverly, MA, USA, model S1Y100) and a peristaltic pump (model CH2, Amicon, inlet flow rate: 2 l/min; inlet and back-pressure : respectively 0.68-0.5 bar (10 and 8 psi)). After a first diafiltration cycle, nanoparticles were diluted in water (2 l) and purification was repeated in order to completely eliminate DMSO and the free protein. The latex suspension was concentrated: final volume of e. g. 100 ml. The suspension was used either immediately or added NaN<sub>3</sub> (0.001 %) and stored at 4°C. It can also be freeze-dried after the addition of the poloxamer: Pluronic F68 (4 wt-%). The total amount of polymer in the final latex suspension was ca. 40 % of the original amount.

[0035] The somatotropin immobilization efficiency was 90%. The total amount of protein immobilized was

measured by using an iodinated (I-125) form of somatotropin and measuring the I-125 associated with the latex. The immobilization efficiency of the active protein was ca. 30 to 40%. Activity of the protein was assessed by radio-immuno-assay (RIA) either directly on the latex suspension, or after lyophilization of the latex dissolution in DMSO, or from the total amount released protein (see next section).

[0036] The rate of the protein released was measured at 12°C by dialysis (membrane of a 100,000 Da cut-off: Spectra/Por Cellulose Ester membrane, Spectrum, Houston, Tx, USA). Nanoparticles were stored in the dialysis bag, and release of the protein was measured against a sodium phosphate buffer (10 mM, pH 7.4) containing serum albumin bovine (1 wt-%) and  $\text{NaN}_3$  (0.02%). This solution was replaced every day (4 ml of latex suspension compared to 6 ml of external buffer). The protein concentration in the external buffer was measured by RIA (active form) and by the amount of I-125 associated with the released iodinated protein (total amount). Kinetics of in vitro release are shown in figure 1. The size of the particles was measured by dynamic light scattering (or PCS) (figure 2).

#### Example 2 (Comparative test)

#### Nanoparticles prepared from poly(D,L)lactide and used to immobilize a somatotropin

[0037] In this case poly (d,l)lactide-co-glycolide was used alone, thus without any biocompatible interacting agents such as in example 1. The mean size of the latex, as analyzed by PCS and transmission electron microscopy (fig. 3) was smaller: 70 nm compared to 100 to 140 nm for the poly(d,l)lactide-co-glycolide/Pluronic® F68 blend. This size has been verified by transmission electron microscopy (figure 3). Figure 4 shows that the protein was released essentially as an inactive compound.

#### Example 3

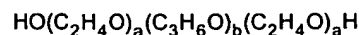
[0038] 225 mg of PLGA, 22,5 mg of poly [HEMA-g-(D, L)-polylactide] graft copolymer, and 25 mg of cholesterol sulfate were dissolved in 50 ml of DMSO (p.a.). Dissolution and oil in water dispersion were performed as described in example 1. As can be seen from figure 5 the protein is also released essentially as an active compound similarly to Example 1.

#### Claims

1. Nanoparticles for proteinaceous drugs or peptides with a particle size between 0.02 to 1  $\mu\text{m}$  containing

a) a biocompatible polymer selected from the group consisting of poly- $\epsilon$ -caprolactone, poly-

lactide, polyglycolide or copolymers thereof,  
b) a biocompatible interacting agent selected from the group consisting of polyethyleneglycol, polyoxyethylene-polyoxypropylene-polyoxyethylene triblock copolymer having the general formula



wherein a and b are selected to give a molecular weight of the total interacting agent in the range of some hundreds to 20,000 Da, or an amphilic graft copolymer containing an hydrophilic backbone grafted with hydrophobic aliphatic polyester subchains,  
c) cholesterol or an anionic cholesterol derivative as biocompatible surfactant,  
d) a peptide and/or a protein as a drug.

wherein substances in a), b) c) and d) form an essentially homogeneous mixture.

2. Nanoparticles according to claim 1, wherein the biocompatible polymer is a polylactide.
3. Nanoparticles according to claim 1 or 2, wherein the drug is an oligo- or polypeptide or a mixture of an oligo- or polypeptide with a low or high molecular weight biologically active substance.
4. Nanoparticles according to claim 1 to 3, wherein the peptide drug is selected from the group consisting of enzymes, antigen molecules, somatotropin, tetanus toxoid, influenza virus.
5. Process for preparing nanoparticles according to claim 1 comprising the steps of
  - mixing the biocompatible polymer and the biocompatible interacting agent in an organic solvent,
  - mixing the solution and evaporating the solvent to form an essentially homogenous mixture or melting one of the components (the biocompatible polymer or the biocompatible interacting agent) and adding the other component (the biocompatible interacting agent or the biocompatible polymer, respectively) so that an essentially homogenous mixture is built up, or melting both components (biocompatible polymer and biocompatible interacting agent) together and forming an essentially homogenous mixture,
  - dissolving the polymer blend in a water soluble organic solvent selected from the group consisting of dimethyl sulfoxide, acetone, tetrahydrofurane, 1,3-dioxolane, N,N-dimethylforma-

- adding cholesterol or an anionic cholesterol derivative as biocompatible surfactant,  
 • adding a polypeptide and/or a protein as drug or drug combination,  
 wherein these steps are carried out in such a manner that the mixture of polymer blend, biocompatible surfactant and drug or drug combination forms an essentially homogenous mixture,  
 • contacting the mixture with an aqueous phase to form a dispersion and  
 • separating the formed nanoparticles from the liquid phase;
6. Process according to claim 5, wherein the separation of nanoparticles and the liquid phase is achieved by filtration, centrifugation or wherein the organic solvent is removed from the nanoparticles by extraction into the aqueous phase and/or ultrafiltration or by evaporation if the organic solvent is sufficiently volatile.
7. Process according to claim 5 or 6, wherein a polymer is added for making easier redispersion of the nanoparticles after freeze-drying of the nanoparticles.
8. Process according to claim 7, wherein the polymer is polyethylene glycol and/or a polyoxyethylene-polyoxypropylene-polyoxyethylene triblock copolymer.
9. Process according to claim 5 to 8, wherein the reaction mixture after partitioning is frozen.
10. Process according to claim 5 to 9, wherein the drug is selected from the group consisting of enzymes, antigen molecules, somatotropin, tetanus toxoid, influenza virus.
11. Use of the nanoparticles according to claims 1 to 4 for the manufacture of a medicament for controlled release drug delivery systems.
12. Use of drugs or drug combinations adhered on or incorporated in nanoparticles of claims 1 to 4 for the manufacture of a medicament for the application to patients in need of the drugs associated with nanoparticles orally, intraperitoneally, topically, intranasally, intravenously in amounts to give an active dose.
- Patentansprüche**
1. Nanopartikel für eiweißähnliche Wirkstoffe oder Peptide mit einer Partikelgröße zwischen 0,02 bis 1 µm, enthaltend
- a) ein bioverträgliches Polymer, ausgewählt aus der Gruppe bestehend aus Poly-ε-caprolacton, Polylactid, Polyglykolid oder Copolymeren davon,  
 b) eine bioverträgliche interagierende Substanz, ausgewählt aus der Gruppe bestehend aus Polyethylenglycol, Polyoxyethylen-Polyoxypropylen-Polyoxyethylen-Triblock-Copolymer mit der allgemeinen Formel
- $$\text{HO}(\text{C}_2\text{H}_4\text{O})_a(\text{C}_3\text{H}_6\text{O})_b(\text{C}_2\text{H}_4\text{O})_a\text{H}$$
- wobei a und b so ausgewählt werden, um ein Molekulargewicht der gesamten interagierenden Substanz im Bereich von mehreren hundert bis 20.000 Da zu ergeben,  
 oder ein amphiphiles Pfropfcopolymer, enthaltend ein hydrophiles Grundgerüst, das mit hydrophoben aliphatischen Polyester-Seitenketten gepfropft ist,  
 c) Cholesterin oder ein anionisches Cholesterinderivat als bioverträglicher grenzflächenaktiver Stoff,  
 d) ein Peptid und/oder ein Protein als Wirkstoff,
- wobei die Substanzen in a), b), c) und d) eine im Wesentlichen homogene Mischung bilden.
2. Nanopartikel nach Anspruch 1, dadurch gekennzeichnet, dass das bioverträgliche Polymer Polylactid ist.
3. Nanopartikel nach Anspruch 1 oder 2, dadurch gekennzeichnet, dass der Wirkstoff ein Oligo- oder Polypeptid oder eine Mischung eines Oligo- oder Polypeptides mit einer niedermolekularen oder hochmolekularen biologisch wirksamen Substanz ist.
4. Nanopartikel nach den Ansprüchen 1 bis 3, dadurch gekennzeichnet, dass der Peptid-Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Enzymen, Antigenmolekülen, Somatotropin, Tetanustoxin, Influenzavirus.
5. Verfahren zur Herstellung von Nanopartikeln, gemäß Anspruch 1, umfassend die Schritte:
- Mischen des bioverträglichen Polymers und der bioverträglichen interagierenden Substanz in einem organischen Lösungsmittel,
  - Mischen der Lösung und Verdampfen des Lösungsmittels, um ein im Wesentlichen homogenes Gemisch zu bilden, oder Schmelzen eines der Bestandteile (das bioverträgliche Polymer

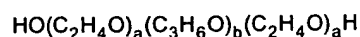
- oder die bioverträgliche interagierende Substanz) und Zufügen der anderen Komponente (die bioverträgliche interagierende Substanz bzw. das bioverträgliche Polymer), so dass ein im Wesentlichen homogenes Gemisch entsteht, oder gemeinsames Schmelzen der beiden Komponenten (das bioverträgliche Polymer oder die bioverträgliche interagierende Substanz) und Bildung eines im Wesentlichen homogenen Gemisches,
- Lösen der Polymermischung in einem in Wasser löslichen organischen Lösungsmittel, ausgewählt aus der Gruppe bestehend aus Dimethylsulfoxid, Aceton, Tetrahydrofuran, 1,3-Dioxolan, N,N-Dimethylformamid oder Mischungen davon,
  - Zugabe von Cholesterin oder eines anionischen Cholesterinderivates als bioverträglichen grenzflächenaktiven Stoff,
  - Zugabe eines Polypeptides und/oder eines Proteins als Wirkstoff oder Wirkstoff-Kombination,
- wobei diese Schritte derart ausgeführt werden, dass das Gemisch aus Polymermischung, bioverträglichem grenzflächenaktiven Stoff und Wirkstoff oder Wirkstoff-Kombination eine im Wesentlichen homogene Mischung bildet,
- Inkontaktbringen der Mischung mit einer wässrigen Phase, um eine Dispersion zu bilden und
  - Trennung der gebildeten Nanopartikel von der flüssigen Phase.
6. Verfahren nach Anspruch 5, **dadurch gekennzeichnet, dass** die Trennung der Nanopartikel und der flüssigen Phase erreicht wird durch Filtration, Zentrifugation oder wobei das organische Lösungsmittel von den Nanopartikeln durch Extraktion in die wässrige Phase entfernt wird und/oder durch Ultrafiltration oder durch Evaporation erreicht wird, wenn das organische Lösungsmittel hinreichend flüchtig ist.
7. Verfahren nach Anspruch 5 oder 6, **dadurch gekennzeichnet, dass** ein Polymer für eine leichtere Redispersion der Nanopartikel nach Gefrier Trocknung der Nanopartikel hinzugefügt wird.
8. Verfahren nach Anspruch 7, **dadurch gekennzeichnet, dass** das Polymer Polyethylenglykol und/oder Polyoxyethylen-Polyoxypropylen-Polyoxyethylen-Triblock-Copolymer ist.
9. Verfahren nach den Ansprüchen 5 bis 8, **dadurch gekennzeichnet, dass** die Reaktionsmischung nach der Abtrennung eingefroren wird.
10. Verfahren nach den Ansprüchen 5 bis 9, **dadurch gekennzeichnet, dass** der Wirkstoff ausgewählt ist aus der Gruppe bestehend aus Enzymen, Antigenmolekülen, Somatotropin, Tetanustoxin, Influenzavirus.
11. Verwendung der Nanopartikel gemäß den Ansprüchen 1 bis 4 zur Herstellung eines Arzneimittels in Form von Wirkstoff-Darreichungs-Systemen mit kontrollierter Freisetzung.
12. Verwendung von Wirkstoffen oder Wirkstoffkombinationen, die an Nanopartikel gemäß der Ansprüche 1 bis 4 anhaften oder in diese Nanopartikel gemäß den Ansprüchen 1 bis 4 eingearbeitet sind, zur Herstellung eines Arzneimittels zur oralen, intraperitonealen, topischen, intranasalen oder intravenösen Verabreichung von mit Nanopartikeln verbundenen Wirkstoffen an Patienten, die die Wirkstoffe benötigen, wobei die Verabreichung in Mengen erfolgt, um eine wirksame Dosis zu ergeben.

#### Revendications

1. Nanoparticules pour des médicaments protéiques ou des peptides ayant une taille de particules comprise entre 0,02 et 1 µm, contenant

a) un polymère biocompatible choisi dans l'ensemble constitué de ε-caprolactone, polylactide, polyglycolide ou des copolymères de ceux-ci,

b) un agent biocompatible d'interaction choisi dans l'ensemble constitué de polyéthylène glycol, copolymère triséquencé de polyoxyéthylène-polyoxypropylène-polyoxyéthylène ayant la formule générale



dans laquelle a et b sont choisis de façon à donner un poids moléculaire de l'agent total d'interaction compris entre quelques centaines et 20 000 Da,

ou un copolymère greffé amphiphile contenant un squelette hydrophile greffé avec des sous-chaînes hydrophobes de polyester aliphatique,

c) du cholestérol ou un dérivé anionique du cholestérol en tant qu'agent tensio-actif biocompatible,

d) un peptide et/ou une protéine en tant que médicament, les substances indiquées en a), b), c) et d) formant un mélange sensiblement homogène.

2. Nanoparticules selon la revendication 1, dans lesquelles le polymère biocompatible est un polylactide.

de.

3. Nanoparticules selon la revendication 1 ou 2, dans lesquelles le médicament est un oligo- ou un polypeptide ou un mélange d'un oligo- ou d'un polypeptide avec une substance biologiquement active de poids moléculaire bas ou élevé.
4. Nanoparticules selon l'un des revendications 1 à 3, dans lesquelles le médicament peptidique est choisi dans l'ensemble constitué par des enzymes, des molécules d'antigènes, la somatotrophine, l'anatoxine tétanique et le virus de la grippe.
5. Procédé de préparation de nanoparticules selon la revendication 1, comprenant les étapes consistant à :
  - mélanger le polymère biocompatible et l'agent biocompatible d'interaction dans un solvant organique,
  - mélanger la solution et évaporer le solvant pour former un mélange sensiblement homogène ou faire fondre l'un des composants (le polymère biocompatible ou l'agent biocompatible d'interaction) et ajouter l'autre composant (respectivement l'agent biocompatible d'interaction ou le polymère biocompatible) de façon à former un mélange sensiblement homogène, ou faire fondre les deux composants (le polymère biocompatible et l'agent biocompatible d'interaction) ensemble et former un mélange sensiblement homogène,
  - dissoudre le mélange polymère dans un solvant organique soluble dans l'eau, choisi dans l'ensemble constitué par le diméthylsulfoxyde, l'acétone, le tétrahydrofurane, le 1,3-dioxolane, le N,N-diméthylformamide ou des mélanges de ceux-ci,
  - ajouter du cholestérol ou un dérivé anionique du cholestérol en tant qu'agent tensio-actif biocompatible,
  - ajouter un polypeptide et/ou une protéine en tant que médicament ou combinaison de médicaments, ces étapes étant effectuées d'une manière telle que le mélange formé du mélange polymère, de l'agent tensio-actif biocompatible et du médicament ou de la combinaison de médicaments forme un mélange sensiblement homogène,
  - amener le mélange au contact d'une phase aqueuse pour former une dispersion et
  - séparer les nanoparticules formées d'avec la phase liquide.
6. Procédé selon la revendication 5, dans lequel on atteint la séparation des nanoparticules et de la

phase liquide par filtration ou par centrifugation, ou dans lequel on élimine le solvant organique des nanoparticules par extraction dans la phase aqueuse et/ou par ultrafiltration ou par évaporation si le solvant organique est suffisamment volatil.

7. Procédé selon la revendication 5 ou 6, dans lequel on ajoute un polymère pour effectuer une redispersion plus aisée des nanoparticules après une lyophilisation des nanoparticules.
8. Procédé selon la revendication 7, dans lequel le polymère est un polyéthylèneglycol et/ou un copolymère triséquencé de polyoxyéthylène-polyoxypropylène-polyoxyéthylène.
9. Procédé selon l'une des revendications 5 à 8, dans lequel on congèle le mélange de réaction après son partage.
10. Procédé selon l'une des revendications 5 à 9, dans lequel le médicament est choisi dans l'ensemble constitué par des enzymes, des molécules d'antigènes, la somatotrophine, l'anatoxine tétanique et le virus de la grippe.
11. Utilisation des nanoparticules selon les revendications 1 à 4 pour la fabrication d'un médicament pour des systèmes d'administration de médicaments à libération maîtrisée.
12. Utilisation de médicaments ou de combinaisons de médicaments adhérent à ou incorporés dans des nanoparticules selon les revendications 1 à 4 pour la fabrication d'un médicament à administrer à des patients nécessitant des médicaments associés à des nanoparticules, par voie orale, intrapéritonéale, topique, nasale ou intraveineuse, en des quantités qui fournissent une dose active.



Fig. 1

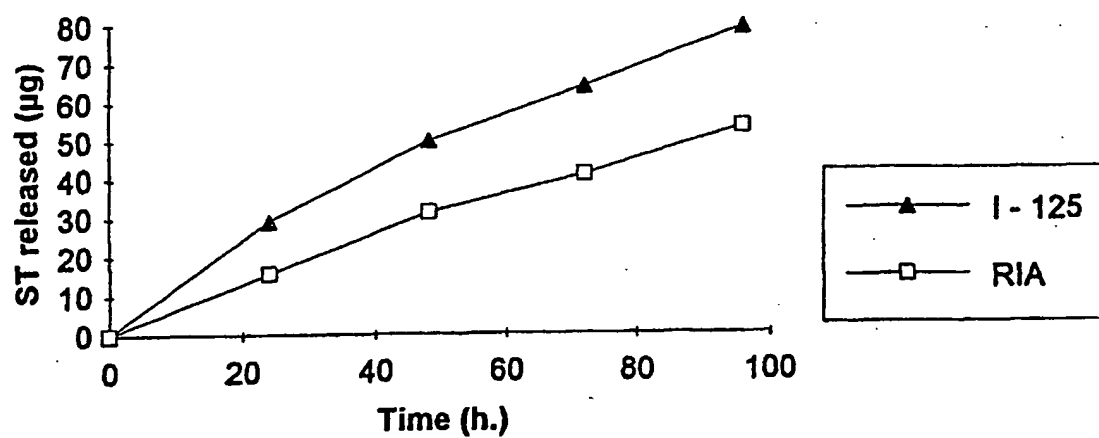


Fig. 2

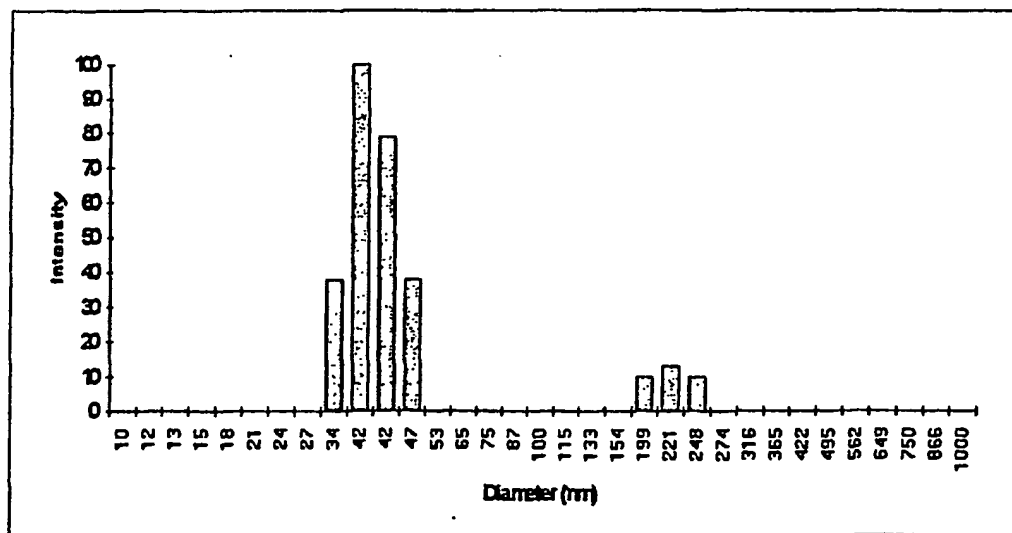


Fig. 3

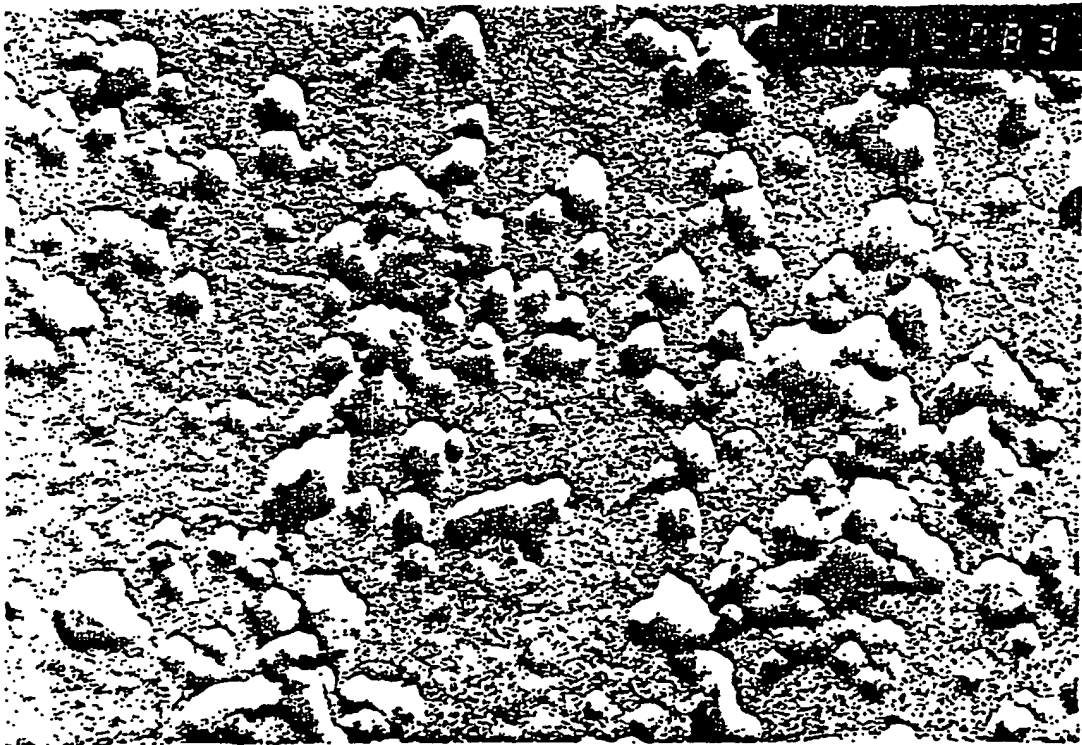


Fig. 4

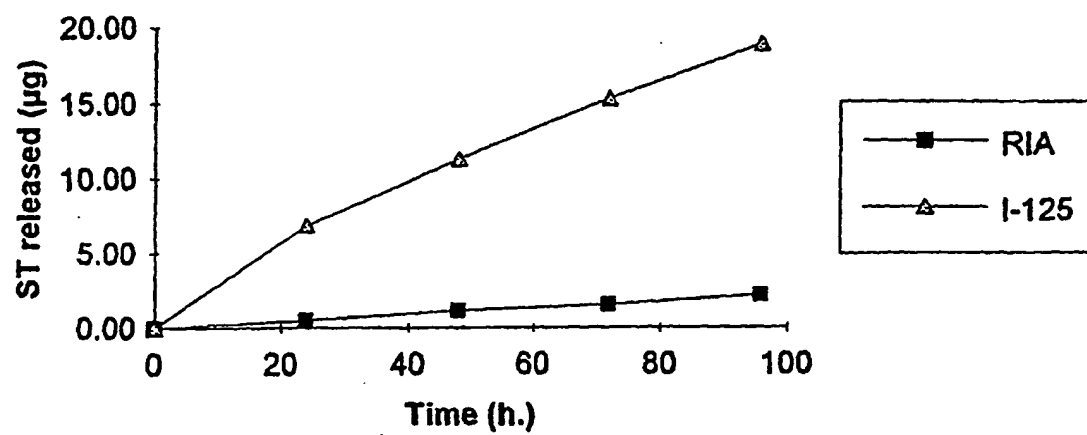


Fig. 5

